Laboratory tomographic microscopy with compact plasma based extreme ultraviolet and soft x-ray sources

Daniel Vicario[1], Alexander von Wezyk[2], Klaus Bergmann[2], Larissa Juschkin[1]
[1]RWTH Aachen University, Chair for Experimental Physics of EUV
[2] Fraunhofer-Institute for Laser Technology

Abstract
In the 'water window' spectral range (wavelength from 2.3 - 4.4 nm), light interaction with biological cells provides a natural contrast used for imaging in x-ray microscopy [1]. The commonly used sources of this radiation are synchrotrons that are expensive with limited accessibility [2]. A compact soft x-ray (SXR) source based on gas-discharge plasma was developed by Bergmann, K. et al. [3, 4] in Fraunhofer Institute for Laser Technology together with RWTH Aachen University. The source produces a highly monochromatic line emission at 2.88 nm, using nitrogen gas, suitable for water window microscopy, see Figure1. The current setup of this microscope based on this source is shown in Figure 2. The setup will incorporate a capillary-type sample holder that allows for 180° rotation of biological samples. Different projections of the samples will be taken and used for reconstruction of tomographic images of biological cells. Currently, the resolution is about 90 nm for the 200 magnification. Sample 2D images of a dried neuron cell are presented below.

Soft X-ray radiation microscopy

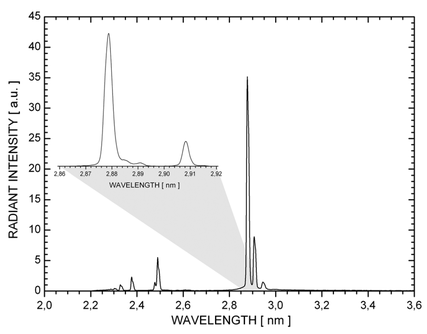


Figure 1: Spectrum at 2.88 nm [5]

The applications of SXR microscopy are:
- micro and nano-structure imaging
- cells and organelles imaging
- identification of the chemistry of proteins and biomolecules

Using nitrogen gas in our compact source the narrow band emission at 2.88 nm can be achieved (see Fig. 1).

Experimental set-up

- Micro zone plate generates a magnified image of the sample on a TE-cooled, back-thinned CCD-detector with 1300x1340 pixels and a pixel size of 20x20 μm^2 (see Fig. 2).
- All optical components are mounted on motorized stages.

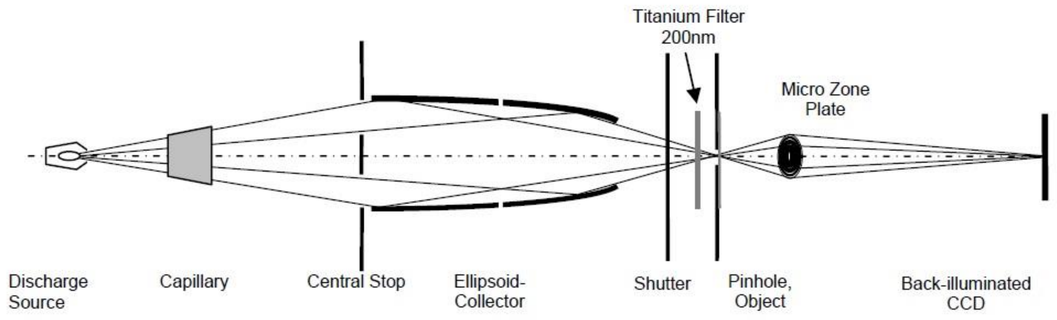


Figure 2: Experimental set-up schematic [2]

Optical parameters of the se-up		
Collector NA_{max} / NA_{min}	at source at sample	0.024 / 0.0126 0.046 / 0.025
Collector focus diameter (FWHM)		x=180 um; y=280 um
Zone plate outer zone width		40 nm
Zone plate NA		0.036

Pulsed gas discharge source

- SXR is produced with a gas discharge source [3] (see Fig. 3).
- Radiance on axis is up to 28 W/(sr cm^2) at a single line at 2.88 nm.
- The current output at the source is $4.12x10^{12}$ photons/(sr pulse) with 1000 µm FWHM emitting diameter.

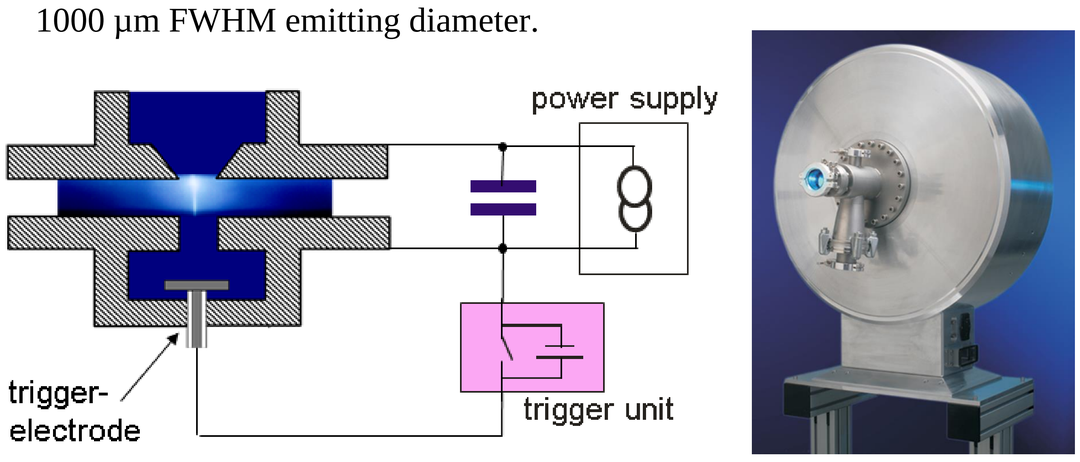


Figure 3: Schematic of the electrode system (left) and the lamp head (right) [4]

Specifications of EUV source for tomographic microscopy	
Working gas	Nitrogen
Gas pressure during operation	5.0×10^{-2} mbar - 7.0×10^{-2} mbar
Voltage during operation	2.0 kV - 3.5 kV
Discharge repetition frequency	2 Hz - 3000 Hz
Capacitor (C)	2.25 µF (9 x 0.25 µF)
Wavelength (λ)	2.88 nm (or 430 eV)

Neuron cell imaging

- For biological cell imaging, a dried cultured mouse hippocampal neuron cell on a 50 nm thick Si_3N_4 membrane was used (see Fig. 4).
- For a magnification of 200, SXR microscope has a better resolution than the light microscope, it also shows contrast of carbon-rich internal structures.

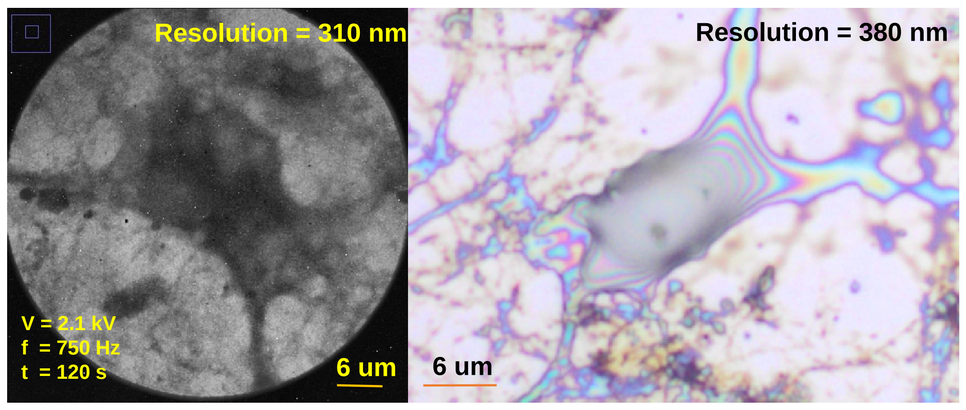


Figure 4: Neuron cell image using SXR microscope (left) and using light microscope(right)

Current magnification and resolution

- For a magnification of 360, the resolution is 90 nm (see Fig. 5).
- Resolution will be increased to 40 to 50 nm by increasing the magnification (750 to 1000 magnification) as previously achieved [1].

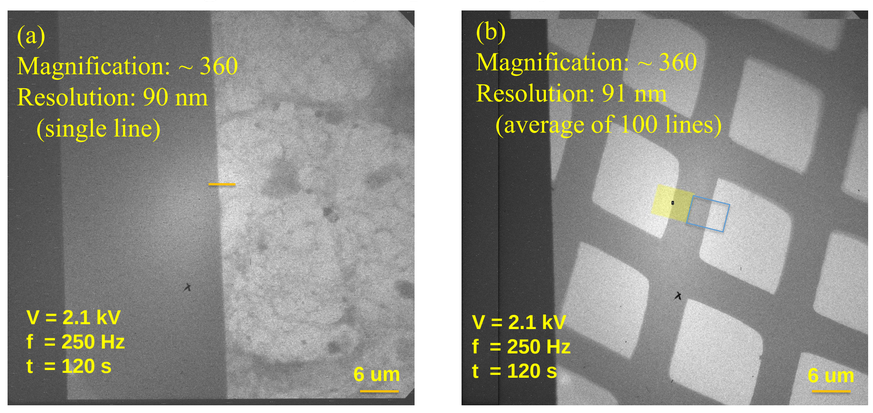
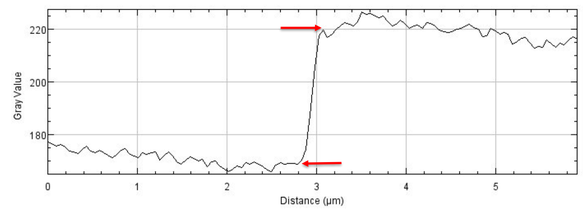


Figure 5: Knife-edge resolution using sample membrane edge (a) and using gold mesh (b and c)



Capillary for biological samples

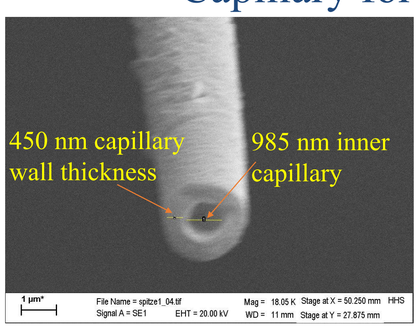


Figure 6: SEM image of the capillary

Quartz capillary:
- less than 0.5 µm tip
- wall thickness of 200 nm to 500 nm
- can contain biological samples from 2 um to 10 um
- transmission of 3 % to 27 % through the sample and the capillary walls

Conclusion

- The resolution of 90 nm is currently limited by pixel size and magnification but already better than the resolution of a visible light microscope.
- The neuron image provides more details and some information on the inner anatomy of the cell.
- The capillaries for biological samples are appropriate for 3D imaging.
- Two-dimensional projection images can be reconstructed to produce a three-dimensional image or tomography image.

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